

## THE ACTION OF LIGHT ON RHODOPSIN\*

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Rhodopsin, a red visual pigment of vertebrate rods, is composed of the yellow carotenoid derivative, retinene, joined to the colorless protein, opsin. It is synthesized by the combination of opsin with a specific hindered *cis* isomer of retinene, neo-*b* (11-*cis*) and bleaches in the light to a mixture of opsin and all-*trans* retinene (cf. Fig. 1). Another *cis* isomer of retinene—iso-*a* (9-*cis*)—combines with opsin to form a second photosensitive pigment, isorhodopsin. Isorhodopsin differs spectroscopically from rhodopsin but bleaches in the light to the identical mixture of all-*trans* retinene and opsin. The synthesis and bleaching of these pigments therefore involve cycles of stereoisomerization of retinene.<sup>1</sup> This seems to be a general situation that involves all the known visual pigments—rhodopsin, iodopsin, porphyropsin, and cyanopsin—and the corresponding isopigments.<sup>2, 3</sup>

If rhodopsin or isorhodopsin is bleached with heat instead of light, the retinene is released in its original *cis* configuration.<sup>4</sup> Stereoisomerization to the all-*trans* configuration is therefore associated not with all bleaching but specifically with bleaching by light.<sup>5</sup>

It has been known for some years that, in the light, rhodopsin bleaches in stages, over intermediates which are short-lived at room temperature, yet stable at low temperatures or in the absence of water.<sup>6, 7</sup> Light alone does not bleach (i.e., decolorize) rhodopsin but converts it to the red photoproduct, lumi-rhodopsin. Lumi-rhodopsin is stable below about  $-45^{\circ}\text{C}$ . Above this temperature, in light or darkness, it goes over to a second orange-red compound, called meta-rhodopsin. In lumi- and meta-rhodopsin the chromophore is still attached to opsin. The major color shift—i.e., bleaching—accompanies the hydrolysis of meta-rhodopsin to retinene and opsin. In light or darkness, vertebrate meta-rhodopsins hydrolyze above about  $-15^{\circ}\text{C}$ ,<sup>7</sup> squid and lobster meta-rhodopsins above about  $+20^{\circ}\text{C}$ .<sup>8, 9</sup> The slow course of this latter reaction strongly suggests that visual excitation precedes the release of retinene and depends upon the light reaction itself or, at most, on the conversion of lumi-rhodopsin to meta-rhodopsin.<sup>10</sup>

On warming meta-rhodopsin in the dark, one obtains a mixture containing roughly one part of retinene and opsin and one part of rhodopsin and isorhodopsin.<sup>7, 11</sup> It was assumed earlier that all these products were formed in the dark, i.e., that meta-rhodopsin in the dark was converted back to rhodopsin and isorhodopsin, in addition to being degraded to retinene and opsin.<sup>7, 11</sup> Recent experiments, however, have clarified the mechanism of the light reaction and necessitate a reinterpretation of these observations.

*Squid rhodopsin.*—Squid rhodopsin does not usually bleach in the light, owing to the relative stability of squid meta-rhodopsin (see above). This enables one to examine squid meta-rhodopsin under more favorable conditions than vertebrate meta-rhodopsins. Thus we have found that, whereas the chromophore of squid rhodopsin, like vertebrate rhodopsin, has the neo-*b* (11-*cis*) configuration, that of meta-rhodopsin is all-*trans*. Thermal bleaching, which does not isomerize the

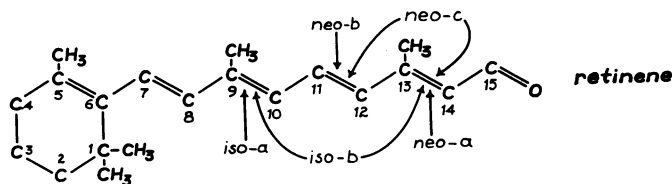


FIG. 1.—Structural formula of retinene, showing the system of numbering carbon atoms. The structure as shown has the all-*trans* configuration. Arrows indicate the double bonds which are in *cis* configuration in the various *cis* isomers.

chromophore (see above), releases predominantly neo-*b* retinene from rhodopsin, but all-*trans* retinene from meta-rhodopsin (Fig. 2).

This is readily demonstrated by irradiating the products of thermal bleaching with white light, which isomerizes retinene to a steady-state mixture of isomers.<sup>1, 12</sup> As shown in Figure 2, the retinene derived from rhodopsin rises in extinction, and  $\lambda_{\max}$  shifts to longer wave lengths, indicating an isomerization predominantly from *cis* to *trans*, whereas the retinene derived from meta-rhodopsin exhibits the opposite behavior. The retinene released by rhodopsin has been further identified as the neo-*b* isomer by its capacity to form rhodopsin upon addition of cattle opsin. The conversion of rhodopsin to meta-rhodopsin therefore involves the isomerization of the chromophore from the neo-*b* to the all-*trans* configuration.

Squid meta-rhodopsin is a pH indicator, red in neutral and mildly acid solution and yellow in alkaline solution. The interconversion of these two forms proceeds readily in the dark and involves only the reversible dissociation of one hydrogen ion from an acid-binding group with pK 7.7.<sup>13</sup> The two forms, called *acid* and *alkaline meta-rhodopsin*, both contain all-*trans* retinene. Their absorption spectra are shown in Figure 3.

Light, having converted rhodopsin to lumi-rhodopsin, can go on to isomerize the all-*trans* chromophore of lumi- or meta-rhodopsin back to the neo-*b* configuration and thus can regenerate rhodopsin. With acid meta-rhodopsin, the isomerization is stereospecific and yields only rhodopsin. The isomerization of alkaline meta-

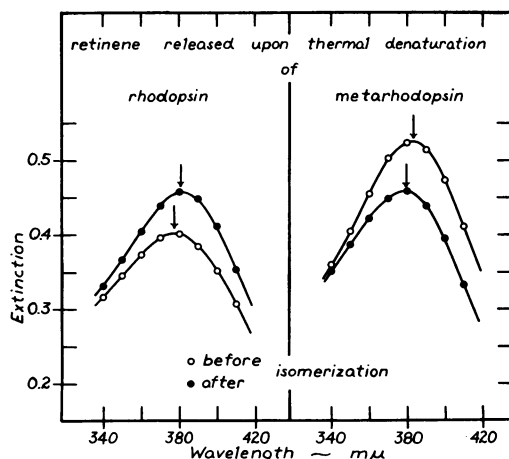
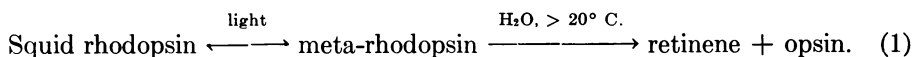


FIG. 2.—Stereoisomeric configurations of retinene released by thermal bleaching of squid rhodopsin (left) and meta-rhodopsin (right) in 2 per cent digitonin, pH 6.3. To minimize thermal isomerization of the products of bleaching (see n. 5), rhodopsin and meta-rhodopsin are denatured by immersing them in a 70° water bath for 30 seconds, and the products chilled immediately on ice. On irradiation with a white isomerizing light (160-watt tungsten-filament lamp with Corning filters 3060 + 3966), the retinene liberated from rhodopsin rises in extinction and  $\lambda_{\max}$  shifts to longer wave lengths (left), whereas the retinene liberated from meta-rhodopsin exhibits opposite changes (right). The results are the same whether acid or alkaline meta-rhodopsin is used.

rhodopsin is less specific, yielding a mixture of rhodopsin and isorhodopsin. Light therefore promotes the interconversion of rhodopsin and meta-rhodopsin, forming steady-state mixtures of both.<sup>13</sup>



The absorption spectra of rhodopsin and acid meta-rhodopsin overlap so widely (cf. Fig. 3) that, starting with either, irradiation with yellow or orange light (Corning filter 3389 or 3482) produces steady-state mixtures containing both pigments in a ratio of about 0.9:1. Since meta-rhodopsin has about 1.5 times the molar extinction of rhodopsin (Fig. 3), the quantum efficiency for isomerizing meta-rhodopsin to rhodopsin (*trans* to *neo-b*) must be about 0.6 (i.e., 0.9/1.5) that of the reverse isomerization.

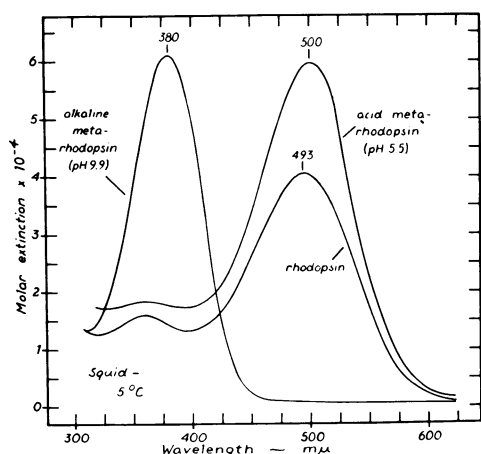


FIG. 3.—Absorption spectra of squid rhodopsin; alkaline meta-rhodopsin produced by irradiating rhodopsin with orange light at pH 9.9; and acid meta-rhodopsin produced by acidification of alkaline meta-rhodopsin to pH 5.5 in the dark. Solvent: 2 per cent aqueous digitonin. For detailed procedure, see n. 13.

Alkaline meta-rhodopsin does not absorb yellow or orange light (cf. Fig. 3) and hence is not isomerized by it. By irradiation with yellow or orange light, squid rhodopsin can therefore be converted quantitatively to alkaline meta-rhodopsin. By acidifying this in the dark, one obtains pure acid meta-rhodopsin—the only way to prepare the pure pigment.<sup>13</sup>

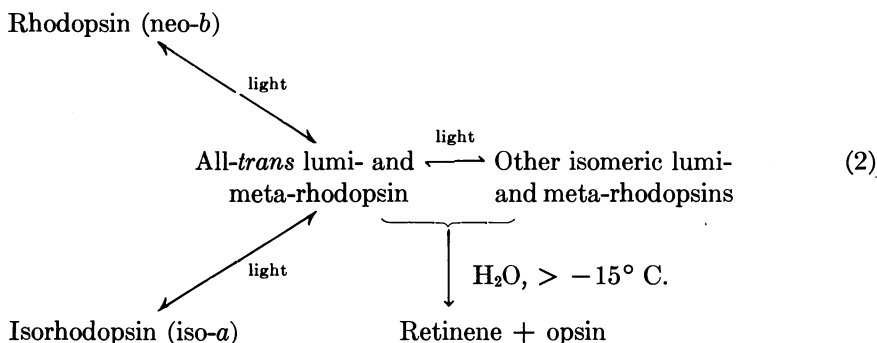
It has been known for some time that retinene in solution is isomerized by simple exposure to light.<sup>1, 12</sup> We see now that light also isomerizes retinene while attached to opsin as the chromophore of squid rhodopsin or meta-rhodopsin. Is this also true for vertebrate rhodopsins?

**Cattle rhodopsin.**—As mentioned above, cattle or frog rhodopsin, after exhaustive irradiation in the cold or in dry films, yields in the dark about half the final product in the form of regenerated rhodopsin and isorhodopsin.<sup>7, 11</sup> It had been assumed earlier that these pigments were generated by a thermal (“dark”) back-reaction from lumi- or meta-rhodopsin. Our experience with squid rhodopsin, however, suggests the following reinterpretation.

The first effect of light upon rhodopsin is to form all-*trans* lumi-rhodopsin, which goes over to all-*trans* meta-rhodopsin by a thermal reaction. Light can go on to isomerize the chromophore of either compound to a steady-state mixture of geometrical isomers. The *neo-b* component of this mixture is rhodopsin; the *iso-a* component is isorhodopsin. No thermal back-reaction is needed to make these substances, for they are regenerated by isomerization *in the light*.

The remaining isomers—all-*trans*, *neo-a*, and so on (cf. Fig. 1)—constitute an isomeric set of lumi- or meta-rhodopsins, all of which are unstable when warmed

in the presence of water and hence bleach to mixtures of retinene and opsin. The reaction sequence can therefore be summarized as follows:



That this is the true situation is demonstrated by the following experiment (Fig. 4). Rhodopsin at  $-17^\circ \text{C}$ . (curve 1) is irradiated exhaustively with white light. Since lumi-rhodopsin is unstable above about  $-45^\circ \text{C}$ ., this yields a product (curve 2) which is identical with what has previously been called "meta-rhodopsin." Its heterogeneity can be demonstrated by bringing it to about pH 13 in the dark, without raising the temperature. Under these conditions, rhodopsin and isorhodopsin bleach slowly (half-lives of about 12 and 5 hours, respectively), and their destruction can be fitted by simple monomolecular decay curves. The product of irradiation, however, does not bleach in this simple manner. The course of its decomposition can be fitted by combining the decay curves of rhodopsin and isorhodopsin with the monomolecular decay curve of a third, much less stable, component with a half-life of about 15 minutes.<sup>14</sup> At the end of 3 hours at pH 13, the solution contains roughly two parts retinene and opsin and one part rhodopsin and isorhodopsin (curve 3). The presence of rhodopsin and isorhodopsin under these rigorous conditions (pH 13,  $-17^\circ \text{C}$ .) strongly suggests their photochemical origin.

The terms "lumi-" and "meta-rhodopsin," as used previously,<sup>7</sup> refer to the product of irradiation (cf. curve 2), hence not to single compounds but to steady-state

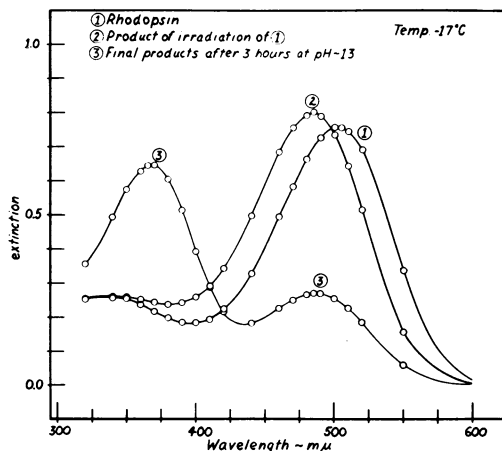


FIG. 4.—Action of light on cattle rhodopsin. Cattle rhodopsin at  $-17^\circ \text{C}$ . (spectrum 1) is irradiated with white light (tungsten-filament lamp, with Jena BG 18 heat filter) until the spectrum stops changing. The product of irradiation (spectrum 2) is not a homogeneous compound, as is shown by raising the pH in the dark from 7 to about 13 with 9 *N* sodium hydroxide. This decomposes part of the mixture. After 3 hours, the solution contains about two parts retinene and opsin, and one part rhodopsin and isorhodopsin (spectrum 3). Solvent: 0.5 per cent digitonin in a 1:1 glycerol-water mixture.

mixtures of chromoproteins including photoregenerated rhodopsin and isorhodopsin. As can be seen from equation (2), we are now redefining lumi- and meta-rhodopsin as the *labile* fraction of the mixture of stereoisomeric chromoproteins, the

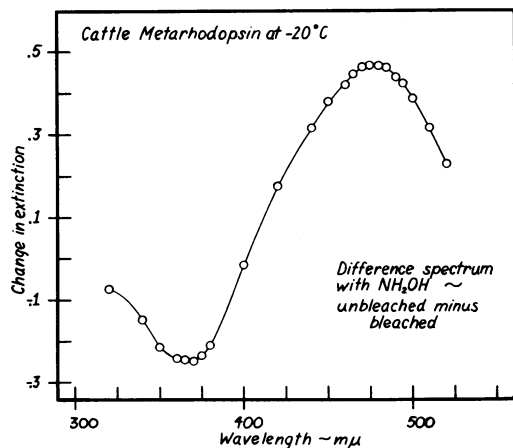


FIG. 5.—Difference spectrum of cattle meta-rhodopsin at  $-20^{\circ}\text{C}$ . Solvent: 0.3 per cent digitonin in a 2:1 glycerol-water mixture. Rhodopsin at  $-20^{\circ}\text{C}$  is irradiated exhaustively with monochromatic light at  $500\text{ m}\mu$ . Hydroxylamine is then added to a final concentration of about  $0.1\text{ M}$  and the spectrum measured. The solution is now warmed to room temperature in the dark. This bleaches the meta-rhodopsin, converting its chromophore to retinene oxime. After about  $\frac{3}{4}$  hour, the solution is recooled to  $-20^{\circ}\text{C}$ , and the spectrum is measured again. The difference between the two is shown. Since retinene oxime has essentially no absorption at wave lengths longer than about  $440\text{ m}\mu$ , above this wave length the difference spectrum is identical with the absorption spectrum.

a steady-state mixture containing more rhodopsin. Analyses of steady-state mixtures produced by irradiation at 450, 500, and  $550\text{ m}\mu$  are shown in Figure 6 and Table 2. As predicted, the mixture produced by irradiation at  $550\text{ m}\mu$  contains about seven times as much meta-rhodopsin as rhodopsin, whereas irradiation at  $450\text{ m}\mu$  yields more nearly equal amounts of the two pigments. The mixture produced at  $500\text{ m}\mu$  is intermediate in composition. The absolute concentration of isorhodopsin is almost the same at all three wave lengths, but this is fortuitous.

From these data one can calculate the relative quantum efficiencies ( $\gamma$ )<sup>16</sup> for the conversion of meta-rhodopsin to rhodopsin and isorhodopsin. The steady-state concentrations of rhodopsin, meta-rhodopsin, and isorhodopsin following irradiation at each wave length are given by the following relationships:<sup>17</sup>

$$(i) \quad \frac{(M)}{(R)} = \frac{\epsilon_r \gamma_{r \rightarrow m}}{\epsilon_m \gamma_{m \rightarrow r}} \quad \text{and} \quad (ii) \quad \frac{(M)}{(I)} = \frac{\epsilon_i \gamma_{i \rightarrow m}}{\epsilon_m \gamma_{m \rightarrow i}},$$

in which  $(R)$ ,  $(M)$  and  $(I)$  are the concentrations of rhodopsin, meta-rhodopsin, and isorhodopsin;  $\epsilon_r$ ,  $\epsilon_m$ , and  $\epsilon_i$ , their molar extinctions at the wave length of irradiation;

fraction which hydrolyzes to retinene and opsin above about  $-15^{\circ}\text{C}$  in the dark. The difference spectrum of cattle meta-rhodopsin so defined is shown in Figure 5.

As one would expect on the basis of this formulation, the experiment shown in Figure 4 yields the same mixtures (spectra 2 and 3), whether one starts with rhodopsin or with isorhodopsin (viz., eq. [2]).

We have pursued the photochemical relationships somewhat further. As can be seen from Table 1, the absorption maxima of rhodopsin, isorhodopsin, and meta-rhodopsin lie at progressively shorter wave lengths. The steady-state mixtures of these compounds should therefore vary somewhat in composition, depending on the wave length of light used to irradiate them. Thus green light, absorbed preferentially by rhodopsin, should favor the formation of meta-rhodopsin, whereas blue light, absorbed preferentially by meta-rhodopsin, would produce

and  $\gamma_r \rightarrow m$ ,  $\gamma_m \rightarrow r$ ,  $\gamma_i \rightarrow m$ , and  $\gamma_m \rightarrow i$ , the quantum efficiencies for their interconversions in the directions indicated by the subscripts.

The molar extinction coefficients of the three compounds are given in Table 3, and their concentrations at the steady state are shown in Figure 6 and Table 2. All quantum efficiencies are expressed relative to the quantum efficiency for converting rhodopsin to meta-rhodopsin ( $\gamma_r \rightarrow m$ ), arbitrarily taken as 1.

TABLE 1\*  
ABSORPTION PROPERTIES OF CATTLE RHODOPSIN, ISORHODOPSIN, AND META-RHODOPSIN AT -20° C. IN 2:1 GLYCEROL-WATER MIXTURE CONTAINING 0.3 PER CENT DIGITONIN

COMPOUND	$\lambda_{\max}$ (m $\mu$ )	MOLAR EXTINCTION AT $\lambda_{\max}$
Rhodopsin.....	502	44,000
Isorhodopsin.....	490	47,700
Meta-rhodopsin.....	478 $\pm$ 2	48,500 $\pm$ 2,000

\* The molar extinctions of rhodopsin and isorhodopsin are calculated from the molar extinctions in this solvent system at room temperature (42,000 and 43,000) by cooling solutions of known extinction to -20° C. and remeasuring their absorption spectra. The data for meta-rhodopsin, averaged from the three experiments shown in Fig. 6, are taken from the difference spectra obtained by subtracting spectra 3 from spectra 2.

TABLE 2\*  
COMPOSITIONS OF STEADY-STATE MIXTURE PRODUCED BY IRRADIATING RHODOPSIN AT -20° C. WITH MONOCHROMATIC LIGHTS OF VARIOUS WAVE LENGTHS (HALF-WIDTH = 11 m $\mu$ )

COMPOUND	STEADY-STATE CONCENTRATIONS FOLLOWING IRRADIATION AT		
	450 m $\mu$	500 m $\mu$	550 m $\mu$
Rhodopsin.....	34	25	11
Isorhodopsin.....	17	17	14
Meta-rhodopsin.....	49	58	75

\* Concentrations, given in percentages, are calculated from the data shown in Fig. 6: rhodopsin and isorhodopsin from spectra 3, by a method similar in principle to one previously described (see R. Hubbard, *J. Gen. Physiol.*, 39, 935, 1955-56); meta-rhodopsin from the differences between the initial concentration of rhodopsin (spectrum 1) and the final concentrations of rhodopsin and isorhodopsin (spectra 3).

TABLE 3\*  
MOLAR EXTINCTION COEFFICIENTS ( $\epsilon$ ) OF CATTLE RHODOPSIN, ISORHODOPSIN, AND META-RHODOPSIN AT 450, 500, AND 550 m $\mu$ .

COMPOUND	MOLAR EXTINCTIONS AT		
	450 m $\mu$	500 m $\mu$	550 m $\mu$
Rhodopsin.....	21,800	43,900	18,600
Isorhodopsin.....	31,100	45,800	9,080
Meta-rhodopsin.....	35,800	40,600	5,400

\* Conditions as described in Table 1.

We can calculate  $\gamma_m \rightarrow r$  from equation (i). In order to calculate  $\gamma_m \rightarrow i$ , however, we need to know  $\gamma_i \rightarrow m$ . This is determined as follows: Rhodopsin and isorhodopsin are bleached with dim white light under conditions in which the light reaction is limiting. The rate of bleaching of each compound is then governed only by the rate at which its chromophore is isomerized to the all-*trans* configuration, as shown in equation (2). Rhodopsin and isorhodopsin absorb white light to about the same extent (within 10 per cent). Under these conditions, their relative rates of bleaching therefore reflect the relative quantum efficiencies for their conversion to all-*trans* meta-rhodopsin. Thus, as seen in Figure 7, we find that the conversion

of isorhodopsin to meta-rhodopsin has a relative quantum efficiency of 0.3. With this value for  $\gamma_{i \rightarrow m}$ , we can calculate  $\gamma_{m \rightarrow i}$  from equation (ii).

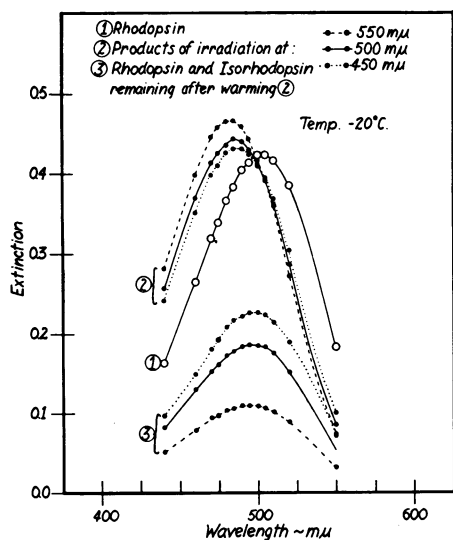


FIG. 6.—Steady-state mixtures of meta-rhodopsin, rhodopsin, and isorhodopsin produced by irradiating cattle rhodopsin at  $-20^{\circ}\text{C}$ . with monochromatic lights of 450, 500, and 550  $m\mu$  (half-width = 11  $m\mu$ ). Rhodopsin (spectrum 1) is irradiated until there is no further change in spectrum (spectra 2). Hydroxylamine is now added to a final concentration of about 0.1  $M$ , and the solutions are warmed to room temperature in the dark to convert meta-rhodopsin to retinene oxime. The solutions are then recooled to  $-20^{\circ}\text{C}$ . and the spectra remeasured and corrected for dilution by hydroxylamine (spectra 3). The three conditions of irradiation yield different mixtures (spectra 2) containing different amounts of rhodopsin and isorhodopsin (spectra 3). The analyses of the mixtures are summarized in Table 2. Solvents as in Fig. 5. These experiments will be described in more detail elsewhere (A. Kropp, in preparation).

The results of these calculations are shown in Table 4. They show that the quantum efficiency for converting rhodopsin to meta-rhodopsin is twice that of the reverse reaction (1:0.5), and the quantum efficiency for converting isorhodopsin to meta-rhodopsin about three times that of the reverse reaction (0.3:0.09).<sup>18</sup>

These experiments have dealt primarily with meta-rhodopsin, although light, in fact, converts rhodopsin to lumi-rhodopsin. What is the difference between lumi- and meta-rhodopsin? Lumi-rhodopsin is stable below about  $-45^{\circ}\text{C}$ . and seems to make a fleeting appearance when rhodopsin or isorhodopsin is irradiated at  $-20^{\circ}\text{C}$ . It is therefore reasonable to assume that lumi-rhodopsin is the initial product of irradiating rhodopsin also at higher temperatures. Its chromophore apparently has the same isomeric configuration as that of meta-rhodopsin, and lumi-rhodopsin can be re-isomerized to rhodopsin and isorhodopsin. The thermal ("dark") reaction which converts lumi-rhodopsin to meta-rhodopsin probably involves a rearrangement of the opsin or of the binding of retinene to opsin, for it does not affect the stereoisomeric configuration of the chromophore.

*Discussion.*—Bleaching, though initiated by the photoisomerization of the

TABLE 4\*

RELATIVE QUANTUM EFFICIENCIES ( $\gamma$ ) FOR CONVERTING CATTLE META-RHODOPSIN TO RHODOPSIN AND ISORHODOPSIN BY IRRADIATION AT 450, 500, AND 550  $m\mu$

WAVE LENGTH OF IRRADIATION ( $m\mu$ )	RELATIVE QUANTUM EFFICIENCIES FOR REACTIONS	
	Meta-Rhodopsin to Rhodopsin	Meta-Rhodopsin to Isorhodopsin
450 .....	0.4	0.09
500 .....	0.5	0.1
550 .....	0.5	0.09
Averages	0.5	0.09

\* Calculated from the data presented in Fig. 6 and Table 2, by means of equations (i) and (ii), as described in the text. The values obtained at the three wave lengths are averaged in the bottom line. However, it remains to be demonstrated whether these quantum efficiencies are independent of wave length and whether it is therefore legitimate to average them.

rhodopsin chromophore to the all-*trans* configuration, is actually effected by a "dark" reaction—the hydrolysis of meta-rhodopsin to retinene and opsin. Light can therefore enter the reaction a second time and *inhibit* bleaching by isomerizing lumi- or meta-rhodopsin back to rhodopsin and isorhodopsin. Thus light, once it has triggered the bleaching of rhodopsin, can go on to reverse this effect.

It has been assumed until now that the quantum efficiency for bleaching rhodopsin, as for all simple photochemical reactions, is independent of light intensity and temperature. This is true at relatively low light intensities.<sup>19</sup> However, since the bleaching of rhodopsin is *not* a simple photochemical reaction, its quantum efficiency cannot under all conditions be that of the initial light reaction—the formation of lumi-rhodopsin. It must involve the probability of further quanta interfering with bleaching by re-isomerizing the chromophores of lumi- or meta-rhodopsin. Obviously, if the rate of delivering quanta (i.e., the light intensity) is so low that meta-rhodopsin is hydrolyzed to retinene and opsin before it can absorb quanta, the quantum efficiency of bleaching will be identical with that of the initial light reaction. However, if the light intensity is increased or the rate of hydrolysis of meta-rhodopsin decreased to the point at which lumi- or meta-rhodopsin begin to absorb quanta, the quantum efficiency of bleaching should decrease. We hope shortly to define the limiting conditions for these effects.

If vision, as is generally believed, is triggered not by the *bleaching* of rhodopsin but by its conversion to lumi- or meta-rhodopsin, the foregoing considerations are not relevant to the excitation process. That is, the quantum efficiency for vision probably is independent of intensity.

Thermal mechanisms for regenerating rhodopsin<sup>15, 20</sup> probably keep pace with its photodecomposition in dim light. In bright light, however, photoregeneration from lumi- and meta-rhodopsin may serve as an additional source of rhodopsin. That a sufficient number of quanta, if delivered within the lifetimes of lumi- and meta-rhodopsin, produce the photosteady state *in vivo*, is suggested by Hagins's observation that in a rabbit's eye a photoflash, "no matter how bright, so long as it lasted less than 1 msec . . . could bleach no more than half the rhodopsin in an

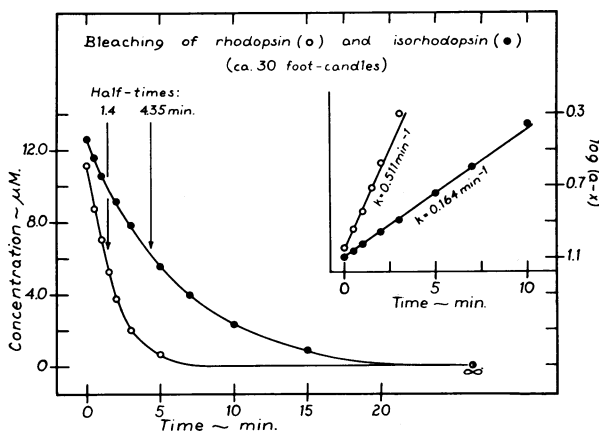


FIG. 7.—Kinetics of photochemical bleaching of cattle rhodopsin and isorhodopsin at 25° C. Solvent: 1.6 per cent aqueous digitonin, 0.2 *M* in hydroxylamine. pH 6.3. The concentrations of rhodopsin and isorhodopsin are plotted as functions of the time of irradiation with dim white light (about 30 foot-candles). In the insert, the same data are replotted in terms of the equation for a monomolecular reaction. Although bleaching involves light and thermal ("dark") reactions, the conditions of this experiment are such that the rate of bleaching is limited by the light reaction. The rate constants (*k*) show that, under identical conditions, rhodopsin bleaches about three times as fast as isorhodopsin.



illuminated area."<sup>21</sup> However, it remains to be established at what intensities this mechanism is activated and hence whether it has physiological significance.

These considerations lead one to wonder why isorhodopsin does not accumulate in the eye.<sup>2</sup> There is no reason to doubt that isorhodopsin will be formed at light intensities which are sufficiently high to support photoregeneration. However, any isorhodopsin which may accumulate during an exposure to bright light will bleach under ordinary conditions of illumination, when the rate of photoregeneration is probably negligible. The only enzymatic isomerization mechanism which has so far been identified yields exclusively the neo-*b* isomer of retinene, hence rhodopsin.<sup>15</sup> We must conclude, therefore, that *in vivo* isorhodopsin will be found only under very special circumstances.

*Summary.*—Rhodopsin is composed of the colorless protein, opsin, combined with the hindered *cis* isomer of retinene, neo-*b* (11-*cis*). Light isomerizes the neo-*b* chromophore, while still attached to opsin, to the all-*trans* configuration, thereby converting rhodopsin to all-*trans* lumi- and meta-rhodopsin. This is probably the reaction responsible for visual excitation. Meta-rhodopsin from lobster and squid is stable below about 20° C., and vertebrate meta-rhodopsins below about -15° C. Above these temperatures, the meta-rhodopsins hydrolyze to retinene and opsin, the reaction responsible for bleaching.

Light, having converted rhodopsin to all-*trans* lumi-rhodopsin, can go on to isomerize the all-*trans* chromophores of lumi- or meta-rhodopsin to steady-state mixtures of stereoisomeric chromophores, all still attached to opsin. The neo-*b* fraction of this mixture constitutes rhodopsin, the iso-*a* fraction isorhodopsin. The other isomers form stereoisomeric sets of lumi- and meta-rhodopsins, all of which hydrolyze in the dark to retinene and opsin. Thus, though it was thought previously that rhodopsin and isorhodopsin were regenerated from meta-rhodopsin by a "dark" reaction, they are, in fact, produced by the isomerization of the chromophores of lumi- and meta-rhodopsin *by light*.

Since light therefore induces not only the bleaching of rhodopsin but also its regeneration, the quantum efficiency of bleaching should decrease under all conditions which favor the photoregeneration of rhodopsin from lumi- or meta-rhodopsin. The quantum efficiency of vision, however, probably depends only on the initial photoisomerization of rhodopsin to all-*trans* lumi-rhodopsin and should not be affected by the photochemical back-reactions.

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<sup>1</sup> R. Hubbard and G. Wald, *J. Gen. Physiol.*, **36**, 269, 1952-53.

<sup>2</sup> There is no evidence that iso-pigments occur *in vivo*.

<sup>3</sup> G. Wald, P. K. Brown, and P. H. Smith, *Science*, **118**, 505, 1953; *J. Gen. Physiol.*, **38**, 623, 1954-55; G. Wald, *Am. J. Ophthalmol.*, **40**, No. 5, 18, 1955.

<sup>4</sup> R. Hubbard, *Nature* (in press).

<sup>5</sup> It has been stated elsewhere (see n. 1) that chemical bleaching of rhodopsin liberates predominantly all-*trans* retinene. Recent experiments in which rhodopsin was bleached with heat show that this is not always the case (see n. 4). Moreover, when determining the configuration of the initial product of bleaching, one must prevent its isomerization after its release from the

chromophore. For example, neo-*b* retinene, liberated by thermal bleaching, isomerizes at the temperatures required for the denaturation of rhodopsin. In order to ascertain its configuration, the rhodopsin must be denatured rapidly (within about 30 seconds) and the product chilled immediately.

<sup>6</sup> R. J. Lythgoe and J. P. Quilliam, *J. Physiol.*, **94**, 399, 1938; G. Wald, *J. Gen. Physiol.*, **21**, 795, 1937-38; E. E. Broda and C. F. Goodeve, *Proc. Roy. Soc. London, B*, **130**, 217, 1941-42.

<sup>7</sup> G. Wald, J. Durell, and R. C. C. St. George, *Science*, **111**, 179, 1950.

<sup>8</sup> R. C. C. St. George and G. Wald, *Biol. Bull.*, **97**, 248, 1949.

<sup>9</sup> G. Wald and R. Hubbard, *Nature*, **180**, 278, 1957.

<sup>10</sup> Hagins (*Nature*, **177**, 989, 1956) has shown that, following flash photolysis of rhodopsin in a rabbit's eye, yellow or orange compounds—presumably retinene—appear before one can record the electroretinographic response to the flash. He therefore concludes that the release of retinene may be the triggering mechanism, after all. Two facts weaken this inference: (1) the appearance of the electroretinogram itself entails a time lag following excitation, and (2) we now know two organisms—lobster and squid—in which retinene is released much more slowly, if at all, under physiological conditions.

<sup>11</sup> F. D. Collins and R. A. Morton, *Biochem. J.*, **47**, 18, 1950.

<sup>12</sup> R. Hubbard, R. I. Gregerman, and G. Wald, *J. Gen. Physiol.*, **36**, 415, 1952-53.

<sup>13</sup> R. Hubbard and R. C. C. St. George, *J. Gen. Physiol.*, **41**, 501, 1957-58.

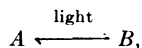
<sup>14</sup> These experiments and calculations will be described in more detail elsewhere (A. Kropp, in preparation).

<sup>15</sup> R. Hubbard, *J. Gen. Physiol.*, **39**, 935, 1955-56.

<sup>16</sup> The quantum efficiency ( $\gamma$ ) is defined as the ratio,

$$\frac{\text{Number of molecules reacting}}{\text{Number of quanta absorbed}}$$

<sup>17</sup> These equations are derived as follows: The rate of a photochemical reaction is given by the product  $\gamma I_{\text{abs}}$ , in which the amount of light absorbed,  $I_{\text{abs}} = I_0 \epsilon c d$ , when  $\epsilon c d < 1$ .  $I_0$  is the intensity of the incident light;  $\epsilon$ , the molar extinction coefficient of the reacting compound;  $c$ , its concentration; and  $d$ , the thickness of the layer of solution which is being irradiated. In a photochemical equilibrium of the type



the rates of the forward and backward reactions are therefore given by the following expressions:

$$\text{Forward rate} = \gamma_A \rightarrow B \epsilon_A I_0 (A) d, \text{ Backward rate} = \gamma_B \rightarrow A \epsilon_B I_0 (B) d,$$

in which  $\gamma_A \rightarrow B$  and  $\gamma_B \rightarrow A$  are the quantum efficiencies for the two reactions;  $\epsilon_A$  and  $\epsilon_B$ , the molar extinctions of *A* and *B*; and  $(A)$  and  $(B)$ , their concentrations. At the photosteady state, the rates of the forward and backward reactions are equal, hence

$$\frac{(B)}{(A)} = \frac{\gamma_A \rightarrow B \epsilon_A}{\gamma_B \rightarrow A \epsilon_B}.$$

It should be noted that this formulation treats meta-rhodopsin as though it were a homogeneous compound, which it probably is not (viz. eq. [2]). This may introduce errors; but, since we do not yet have adequate methods with which to analyze meta-rhodopsin into its isomeric components, much less measure their rates of interconversion, we must for the present be content with this approximation.

<sup>18</sup> We should like to draw attention to the fact that the relative rates of isomerization of the three types of chromophores parallel those of the respective isomers of retinene in solution. Thus, upon irradiation, all *trans* retinene yields neo-*b* retinene faster than iso-*a* retinene (see R. Hubbard, *J. Gen. Physiol.*, **39**, 935, 1955-56), and meta-rhodopsin is converted to rhodopsin more rapidly than to isorhodopsin. Likewise, neo-*b* retinene is isomerized faster than iso-*a* retinene (see R. Hubbard, *J. Am. Chem. Soc.*, **78**, 4662, 1956), and rhodopsin is converted to meta-rhodopsin more rapidly than is isorhodopsin.

<sup>19</sup> S. Hecht, *J. Gen. Physiol.*, **3**, 286, 1920-21; *ibid.*, **6**, 731, 1923-24. However, Dartnall, Good-eve, and Lythgoe (*Proc. Roy. Soc. London, A*, **164**, 216, 1938) report a somewhat lower quantum yield for bleaching at 5° than at 20°-60° C.

<sup>20</sup> F. D. Collins, J. N. Green, and R. A. Morton, *Biochem. J.*, **53**, 152, 1953; *ibid.*, **56**, 493, 1954.

<sup>21</sup> W. A. Hagins, *J. Physiol.*, **129**, 22P, 1955.